

## **Exploiting The Substrate Promiscuity of Hydroxycinnamoyl-CoA:shikimate Hydroxycinnamoyl Transferase to Reduce Lignin**

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**Project Goals: The most abundant organic material on earth is lignocellulosic biomass or non-food plant fiber. JBEI's mission is to convert biomass to biofuels. The goal is to provide the nation with clean, renewable transportation fuels identical to gasoline, diesel and jet fuel. Inside JBEI's Emeryville laboratories, researchers are using the latest tools in molecular biology, chemical engineering, computational and robotic technologies, and pioneering work in synthetic biology to transform biomass sugars into energy-rich fuels. One of the goals of the cell wall engineering team in JBEI's Feedstocks Division is to engineer lignin in plants for reducing biomass recalcitrance.**

Lignin poses a major challenge in the processing of plant biomass for agro-industrial applications. For bioengineering purposes, there is a pressing interest in identifying and characterizing the enzymes responsible for the biosynthesis of lignin [1]. Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT, EC 2.3.1.133) is a key metabolic entry point for the synthesis of the most important lignin monomers: coniferyl and sinapyl alcohols [2]. In this study, we investigated the substrate promiscuity of HCT from a bryophyte (*Physcomitrella*) and from five representatives of vascular plants (*Arabidopsis*, poplar, switchgrass, pine, and *Selaginella*) using a yeast expression system. We demonstrate for these HCTs a conserved capacity to acylate with *p*-coumaroyl-CoA several phenolic compounds in addition to the canonical acceptor shikimate normally used during lignin biosynthesis. Using either recombinant HCT from switchgrass (PvHCT2a) or *Arabidopsis* and switchgrass stem protein extracts, we show evidence of the inhibitory effect of these phenolics on the synthesis of *p*-coumaroyl shikimate in vitro, which presumably occurs via a mechanism of competitive inhibition. Structural study of PvHCT2a confirmed the binding of a non-canonical acceptor in a similar manner as shikimate in the active site of the enzyme. Finally, we exploited in *Arabidopsis* the substrate flexibility of HCT to reduce lignin content and improve biomass saccharification by engineering transgenic lines that overproduce one of the HCT non-canonical acceptors. Our results demonstrate conservation of HCT substrate promiscuity and provide support for a new strategy for lignin reduction in the effort to improve the quality of plant biomass for forage and cellulosic biofuels.

This work is part of the DOE Joint BioEnergy Institute supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy.